

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claim in the application.

Please note that this listing of the claims contains no new amendments.

1. (Previously presented) A method for extending an oligonucleotide primer or a pair of oligonucleotide primers using an enzymatic cycle primer extension reaction at temperatures between about 45°C and about 65°C, and a melting temperature of about 70°C, comprising the step of mixing a template DNA with a primer or a pair of primers and a natural or a modified form of a moderately thermostable DNA polymerase from an organism selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, wherein the DNA polymerase has proofreading 3'-5' exonuclease activity during DNA primer extension over a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template, in a solution containing between about 10% and about 20% (v/v) glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol, under conditions that the cycle reaction temperature fluctuates between a melting temperature of about 70°C, and an annealing temperature of about 37°C, and repeating the cycle primer extension reaction at least two times and until the DNA polymerase repeatedly extends the primer or pair of primers to produce a sequence-specific amplification product of a desired length.

2. (Previously presented) The method of claim 1, wherein the glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol is present in about 15% (v/v).

3. (original) The method of claim 1, wherein the DNA polymerase has an optimum enzymatic activity at about 65°C.

4. (original) The method of claim 1, wherein the DNA polymerase has an amino acid sequence that shares not less than 95% homology of a DNA polymerase isolated from *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus*.

5. (canceled) The method of claim 1, which comprises the further step of repeating the cycle primer extension reaction.

6. (original) The method of claim 1, wherein copies of a selected segment of a double-stranded DNA are amplified in the presence of a forward primer and a reverse primer to the template by repeated heating and cooling cycles.

7. (original) The method of claim 6, wherein the forward primer and reverse primer may be of varying lengths.

8. (Previously presented) The method of claim 1, wherein oligonucleotide primers of various lengths are extended with specific nucleotide terminations in the presence of ddNTPs or their analogs for cycle sequencing.

9. (Previously presented) A method for extending an oligonucleotide primer annealed to a DNA template for direct cycle sequencing of in vitro amplified double-stranded DNA products without prior isolation or purification, using an enzymatic cycle primer extension reaction at temperatures between about 45°C and about 65°C, and a melting temperature below about 80°C, comprising the steps of:

- (i) mixing diluted crude amplified reaction product with an excess amount of a sequencing primer, the four standard ddNTP terminators or their corresponding analogs, a native or modified form of a moderately thermostable DNA polymerase selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, wherein

the DNA polymerase has proofreading 3'-5' exonuclease activity during DNA primer extension over a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template, a suitable concentration of dNTPs, and a composition comprising a buffer in a solution containing about 10% to about 20% of glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol, and

- (ii) effecting cycle primer extension reaction(s) at a temperature below about 80°C for at least two times to extend the sequencing primer molecules to desired lengths terminated specifically by ddNTPs or their corresponding analogs and thereby produce a sequence-specific amplification product of a desired length.

10. (original) The method of claim 9, wherein in vitro amplified double-stranded DNA products are generated by extending a primer or a pair of primers using an enzymatic cycle primer extension reaction at temperatures below about 80°C, comprising the step of mixing a target segment of DNA with a primer or a pair of primers and a natural or a modified form of a moderately thermostable DNA polymerase from an organism selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, in a solution containing about 10% to about 20% (v/v) glycerol, ethylene glycol, or a mixture thereof, under conditions that the cycle reaction temperature fluctuates between a melting temperature of about 70°C and a cooling temperature of about 37°C, so that the DNA polymerase repeatedly extends the primer or pair of primers.

11. (original) The method of claim 9, wherein the moderately thermostable DNA polymerase has an amino acid sequence that shares not less than 95% homology of a

DNA polymerase isolated from *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus*,

Claims 12-17. (canceled)

18. (Previously presented) The method according to claim 1, wherein the DNA polymerase has at least 95% homology to the amino acid sequence of SEQ ID NO:2.

19. (Previously presented) The method according to claim 1, wherein the DNA polymerase is expressed from a DNA sequence that has at least 95% homology to the DNA sequence of SEQ ID NO:1.

20. (Previously presented) The method according to claim 1, wherein the DNA polymerase has the amino acid sequence of SEQ ID NO:2.

21. (Previously presented) The method according to claim 1, wherein the DNA polymerase is expressed from a DNA sequence having the DNA sequence of SEQ ID NO:1.

22. (Previously presented) A method for extending an oligonucleotide primer or a pair of oligonucleotide primers using an enzymatic cycle primer extension reaction at temperatures between about 45°C and about 65°C, and a melting temperature of about 70°C, comprising the step of mixing a template DNA with a primer or a pair of primers and a natural or a modified form of a moderately thermostable DNA polymerase from an organism selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, wherein the DNA polymerase during dye-labeled terminator automated DNA cycle sequencing reduces the innate selective discrimination against the incorporation of fluorescent dye-labeled ddCTP and fluorescent dye-labeled ddATP, in a solution containing between about 10% and about 20% (v/v) glycerol,

ethylene glycol, or a mixture of glycerol and ethylene glycol, under conditions that the cycle reaction temperature fluctuates between a melting temperature of about 70°C and an annealing temperature of about 37°C, and repeating the cycle primer extension reaction at least two times and until the DNA polymerase repeatedly extends the primer or pair of primers to produce a sequence-specific amplification product of a desired length.

23. (Previously presented) The method of claim 22, wherein the DNA polymerase has proofreading 3'-5' exonuclease activity during DNA primer extension over a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template.

24. (Previously presented) The method according to claim 22, wherein the DNA polymerase has at least 95% homology to the amino acid sequence of SEQ ID NO:4.

25. (Previously presented) The method according to claim 22, wherein the DNA polymerase is expressed from a DNA sequence that has at least 95% homology to the DNA sequence of SEQ ID NO:3.

26. (Previously presented) The method according to claim 22, wherein the DNA polymerase has the amino acid sequence of SEQ ID NO:4.

27. (Previously presented) The method according to claim 22, wherein the DNA polymerase is expressed from a DNA sequence having the DNA sequence of SEQ ID NO:3.

28. (Previously presented) The method of claim 22, wherein the glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol is present in about 15% (v/v).

29. (Previously presented) The method of claim 22, wherein oligonucleotide primers of various lengths are extended with specific nucleotide terminations in the presence of ddNTPs or their analogs for cycle sequencing.

30. (Previously presented) A method for extending an oligonucleotide primer annealed to a DNA template for direct cycle sequencing of in vitro amplified double-stranded DNA products without prior isolation or purification, using an enzymatic cycle primer extension reaction at temperatures between about 45°C and about 65°C, and a melting temperature below about 80°C, comprising the steps of:

- (i) mixing diluted crude amplified reaction product with an excess amount of a sequencing primer, the four standard ddNTP terminators or their corresponding analogs, a native or modified form of a moderately thermostable DNA polymerase selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, wherein the DNA polymerase during dye-labeled terminator automated DNA cycle sequencing reduces the innate selective discrimination against the incorporation of fluorescent dye-labeled ddCTP and fluorescent dye-labeled ddATP, a suitable concentration of dNTPs, and a composition comprising a buffer in a solution containing about 10% to about 20% of glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol, and
- (ii) effecting cycle primer extension reaction(s) at a temperature below 80°C for at least two times to extend the sequencing primer molecules to desired lengths terminated specifically by ddNTPs or their corresponding analogs and thereby produce a sequence-specific amplification product of a desired length.

31. (Previously presented) The method of claim 30, wherein the DNA polymerase has proofreading 3'-5' exonuclease activity during DNA primer extension over a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template.

32. (Previously presented) The method according to claim 30, wherein the DNA polymerase has at least 95% homology to the amino acid sequence of SEQ ID NO:4.

33. (Previously presented) The method according to claim 30, wherein the DNA polymerase is expressed from a DNA sequence that has at least 95% homology to the DNA sequence of SEQ ID NO:3.

34. (Previously presented) The method according to claim 30, wherein the DNA polymerase has the amino acid sequence of SEQ ID NO:4.

35. (Previously presented) The method according to claim 30, wherein the DNA polymerase is expressed from a DNA sequence having the DNA sequence of SEQ ID NO:3.